ORIGINAL ARTICLE

CHARGE syndrome: the phenotypic spectrum of mutations in the *CHD7* gene

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Supplemental tables are available at http://www.jmedgenet.com/supplemental

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Background: CHARGE syndrome is a non-random clustering of congenital anomalies including coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies, and deafness. A consistent feature in CHARGE syndrome is semicircular canal hypoplasia resulting in vestibular areflexia. Other commonly associated congenital anomalies are facial nerve palsy, cleft lip/palate, and tracheo-oesophageal fistula. Specific behavioural problems, including autistic-like behaviour, have been described. The *CHD7* gene on chromosome 8q12.1 was recently discovered as a major gene involved in the aetiology of this syndrome.
Methods: The coding regions of *CHD7* were screened for mutations in 107 index patients with clinical features suggestive of CHARGE syndrome. Clinical data of the mutation positive patients were sampled to study the phenotypic spectrum of mutations in the *CHD7* gene.
Results: Mutations were identified in 69 patients. Here we describe the clinical features of 47 of these patients, including two sib pairs. Most mutations were unique and were scattered throughout the gene. All patients but one fulfilled the current diagnostic criteria for CHARGE syndrome. No genotype-phenotype correlations were apparent in this cohort, which is best demonstrated by the differences in clinical presentation in sib pairs with identical mutations. Somatic mosaicism was detected in the unaffected mother of a sib pair, supporting the existence of germline mosaicism.
Conclusions: *CHD7* mutations account for the majority of the cases with CHARGE syndrome, with a broad clinical variability and without an obvious genotype-phenotype correlation. In one case evidence for germline mosaicism was provided.

CHARGE syndrome (OMIM 214800) is a pleiotropic disorder comprising of *c*oloboma, *h*eart defects, choanal *a*tresia, *r*etarded growth and development, *g*enital hypoplasia, *e*ar anomalies, and deafness. A consistent feature in CHARGE syndrome is semicircular canal hypoplasia resulting in vestibular areflexia.[1–3] Other commonly associated congenital anomalies are facial nerve palsy, cleft lip/palate, and tracheo-oesophageal fistula. Specific behavioural problems, including autistic-like behaviour, have been described.[4 5] The combination of abnormalities initially known as CHARGE association was first reported independently by both Hall and Hittner *et al* in 1979,[6 7] after which Pagon and colleagues proposed the acronym CHARGE in 1981.[8] CHARGE syndrome is an autosomal dominant syndrome with an estimated prevalence at birth between 1 per 10 000 and 1 per 15 000.[9] Recent epidemiological data revealed the occurrence of CHARGE syndrome in 1 in 8500 live births in the Atlantic Provinces of Canada.[10]

CHARGE syndrome is a phenotypically heterogeneous syndrome clinically diagnosed using criteria which have been refined several times. Blake *et al* suggested diagnostic criteria in 1998.[9] A refinement of these criteria for different age groups was proposed to capture the continuum of the presentation of CHARGE syndrome.[10] Simultaneously, Verloes suggested an update of diagnostic criteria, emphasising the most specific embryological defects while avoiding non-specific or secondary anomalies.[11] He also suggested the exclusion of sex dependent criteria. Both sets of diagnostic criteria are given in table 1.

CHARGE syndrome was only recently reconsidered to be a syndrome instead of an association after our group discovered *CHD7* on chromosome 8 (8q12.1) as a major gene involved in this syndrome.[12] *CHD7* encodes a protein of the chromodomain (*chr*omatin *o*rganisation *mo*difier) family. Members of this family share a unique combination of functional domains consisting of two N-terminal *c*hromodomains, followed by a SWI2/SNF2-like ATPase/*h*elicase domain and a *D*NA binding domain.[13 14] It is assumed that CHD protein complexes affect chromatin structure and gene expression and, thereby, play an important role in regulating embryonic development.

We report a study of the phenotypic spectrum in 47 patients with a *CHD7* mutation, with special emphasis on differences in presentation in sib pairs that share identical mutations.

METHODS

Patients

The coding regions of the *CHD7* gene were screened for mutations in 107 index patients with clinical features suggestive of CHARGE syndrome. In 69 of these patients a mutation was identified (65%), and for 47 patients (22 males, 25 females, two sib pairs) sufficient clinical data were

Table 1 Updated diagnostic criteria for CHARGE syndrome

Verloes[11] (typical CHARGE = 3 major criteria or 2 major and 2 minor criteria)	Blake *et al*[9] (all 4 major criteria, or 3 major and 3 minor criteria)
Major criteria	Major criteria
1) Coloboma (iris or choroid, with or without microphthalmia)	1) Coloboma (iris, retina, choroid, optic disc, or microphthalmia)
2) Atresia of choanae	2) Atresia of choanae
3) Hypoplastic semicircular canals	3) Cranial nerve dysfunction - I: anosmia, VII: facial palsy, VIII: sensorineural deafness and vestibular problems, IX and/or X: swallowing problems
	4) Characteristic external ears (absent or hypoplastic lobes, asymmetry, decreased cartilaginous folds, and triangular concha) and inner ear anomalies (temporal bone findings with cochlear hypoplasia and or absent/hypoplastic semicircular canals)
Minor criteria	Minor criteria
1) Rhombencephalic dysfunction (brainstem dysfunctions, cranial nerve VII to XII palsies, and neurosensory deafness)	1) Characteristic facial features - broad, sloping forehead, laterally protruding ears, small mouth, and high nasal bridge
2) Malformation of mediastinal organs (heart, oesophagus)	2) Congenital cardiovascular malformations of all types
3) Hypothalamo-hypophyseal dysfunction (including GH and gonadotrophin deficiencies)	3) Tracheo-oesophageal fistula
4) Abnormal middle or external ear	4) Growth deficiency
5) Mental retardation	5) Genital hypoplasia - micropenis and/or cryptorchidism or hypoplastic labia. Delayed, incomplete pubertal development
	6) Orofacial cleft
	7) Developmental delay: delayed motor milestones, hypotonia, mental retardation

available to include them in further studies. The cohort includes 15 patients reported in our previous study.[12] Parental DNA samples of 22 patients, including one sib pair, were tested for de novo occurrence.

Clinical information concerning the patients was obtained through investigation in our own department or through a written questionnaire submitted when DNA of the patient was referred to the DNA diagnostics section of our department. Additional information was obtained from clinicians when necessary. The diagnostic criteria of Blake and Verloes (table 1) were applied to all cases for which sufficient clinical information was available.[9 11]

All patients or their legal representatives gave informed consent for the DNA studies and the collection of clinical data.

Mutation screening

DNA was isolated according to standard procedures. The 37 coding exons of the *CHD7* gene (exons 2–38, accession number NM_017780) and their flanking intron sequences were amplified by polymerase chain reaction (PCR). Subsequently, sequence analysis was performed using a 3730 automated sequencer (Applied Biosystems, Foster City, CA).

The primer sets used previously were optimised by using shorter PCR products to exclude allele dropout.[12] Primer information and PCR conditions are given in supplemental tables I and II, available at http://www.jmedgenet.com/supplemental.

Whole gene deletions were excluded by multiplex ligation dependent probe amplification (MLPA). Specific probe sets were designed for exons 2–11 and exons 33–38. MLPA analysis was performed according to the instructions of the manufacturer (MRC-Holland, Amsterdam, the Netherlands; www.mlpa.com). Probe information is given in supplemental table 3 available at http://www.jmedgenet.com/supplemental.

RESULTS

CHD7 mutation analysis

Mutation analysis in our series of 107 index patients revealed 69 mutations in the *CHD7* gene (fig 1 and table 2). Two mutations were recurrent, and all others were unique. We detected 31 nonsense, 17 frame shift, 13 splice site, and 8 missense mutations scattered throughout the gene. In the affected sibs identical mutations were identified. A female *CHD7* positive patient (no. 22) had a previously identified chromosome 22q11 deletion. Fifteen patients were reported in a previous study,[12] and in six of them the mutation was not detected initially. However, after a more thorough investigation with improved primer sets, *CHD7* mutations were detected in these patients. The parents were studied for 21 index patients. In 20 cases, the mutation was proven to be de novo. In the sib pair consisting of two boys, mosaicism for the *CHD7* mutation was identified in the mother. In the remaining 38 mutation negative patients, whole gene deletions were excluded by MLPA analysis.

Clinical features

Information obtained through our own investigation and/or through written questionnaires, supplemented with

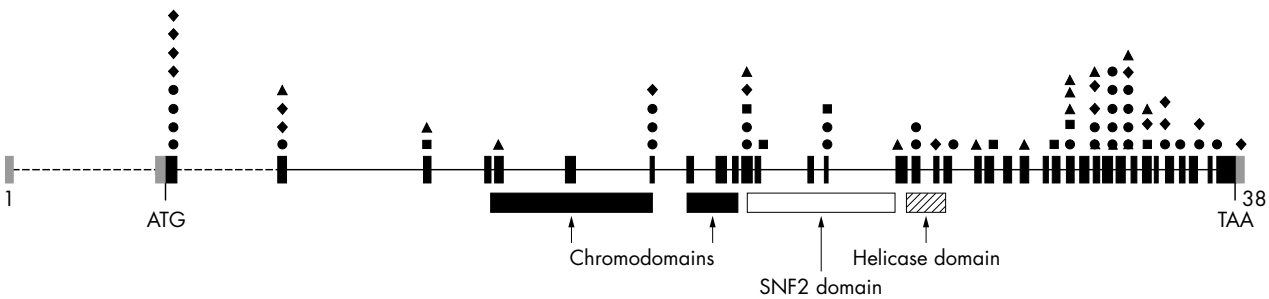

Figure 1 Distribution of *CHD7* mutations identified in the 69 CHARGE syndrome patients. Coding exons are indicated in black bars, whereas the non-coding sequences are indicated in grey. Mutations are schematically shown above the exons in which they are located. Nonsense mutations are represented by ● (n = 31), missense mutations by ■ (n = 8), frameshift mutations by ◆ (n = 17), and splice site mutations by ▲ (n = 13), respectively.

Table 2 Overview of *CHD7* mutations

Mutation in *CHD7*	Exon	Theoretical effect on RNA (r.) or protein (p.)*	Segregation	Patient in tables 3 and 4
c.77_78delAA	2	p.Glu26fs	ND	1
c.469C>T	2	p.Arg157X	ND	
c.921_922delAG (e2)	2	p.Gly308fs	ND	
c.1044delC	2	p.Asn349fs	ND	2
c.1078G>T	2	p.Gly360X	One parent excl.	3
c.1388delG	2	p.Gly463fs	De novo	4
c.1465C>T	2	p.Gln489X	ND	5
c.1495C>T	2	p.Gln499X	ND	
c.1714C>T	3	p.Gln572X	De novo	6
c.1973_1974insT	3	p.Glu658fs	ND	7
c.2095A>G	3	r.spl? p.S699G	ND	
c.2194C>G	4	p.Pro732Ala	ND	
c.2238+1G>A	IVS4	r.spl?	De novo	8
c.2442+5G>A	IVS6	r.spl?	ND	9
c.2504_2508delATCTT	8	p.Tyr835fs	ND	
c.2505T>A	8	p.Tyr835X	De novo	10
c.2520G>A	8	p.Trp840X	ND	
c.2572C>T	8	p.Arg858X	De novo	11
c.2958-2A>T	IVS11	r.spl?	De novo	12
c.2959C>T	12	p.Arg987X	ND	13
c.3053_3054insA	12	p.Phe1019fs	ND	14
c.3082A>G	12	p.Ile1028Val	De novo	45
c.3106C>T	12	p.Arg1036X	ND	
c.3302G>A	13	p.Cys1101Tyr	ND	
c.3654C>G	15	p.Tyr1218X	ND	
c.3655C>T	15	p.Arg1219X	ND	15
c.3770T>G	15	p.Leu1257Arg	De novo	46
c.3779-2A>G	IVS15	r.spl?	ND	16
c.4015C>T	17	p.Arg1339X	ND	
c.4157C>G	17	p.Ser1386X	One parent excl.	17
c.4226_4227delTG	18	p.Val1409fs	ND	18
c.4507G>T	19	p.Glu1503X	ND	19
c.4644+1G>A	IVS20	r.spl?	ND	
c.4787A>G	21	p.Asp1596Gly	ND	
c.5050-41_5050-3del39	IVS23	r.spl?	ND	20
c.5402A>C	25	p.His1801Pro	De novo	47
c.5405-17G>A	IVS25	r.spl?	De novo	21
c.5405-7G>A	IVS25	r.spl?	ND	
c.5418C>G	26	p.Asn1807X 22q11del	De novo	22
c.5436C>A	26	p.Asp1812Glu	ND	
c.5534G>A	IVS26	r.spl?	De novo	23
c.5668A>T	29	p.Lys1890X	De novo	24
c.5680_5681delAG	29	p.Ser1894fs	De novo	25
c.5752_5753dupA	29	p.Thr1918fs sib pair 1	ND	26
c.5752_5753dupA	29	p.Thr1918fs sib pair 1	ND	27
c.5833C>T	29	p.Arg1945X	De novo	28
c.5893+1G>A	IVS29	r.spl?	ND	
c.5982G>A	30	p.Trp1994X†	ND	
c.5982G>A	30	p.Trp1994X† sib pair 2	Mat. mosaicism	29
c.5982G>A	30	p.Trp1994X† sib pair 2	Mat. mosaicism	30
c.6051T>A	30	p.Cys2017X	ND	31
c.6070C>T	30	p.Arg2024X	One parent excl.	32
c.6079C>T	30	p.Arg2027X	De novo	33
c.6148C>T	31	p.Arg2050X†	ND	35
c.6148C>T	31	p.Arg2050X†	ND	34
c.6155_6157CTC>AGA	31	p.Ser2052X	ND	
c.6157C>T	31	p.Arg2053X	ND	
c.6304delG	31	p.Val2102fs	De novo	36
c.6775+2_6775+3insGT	IVS31	r.spl?	ND	37
c.6955C>T	33	p.Arg2319Cys	ND	
c.7079delA	33	p.Lys2360fs	ND	
c.7165-4A>G	IVS33	r.spl? p.Lys2388_Glu2389insX	ND	
c.7180delC	34	p.Lys2394fs	ND	38
c.7219delA	34	p.Ile2407fs	ND	
c.7252C>T	34	p.Arg2418X	ND	39
c.7400delT	34	p.Leu2467fs	De novo	40
c.7824T>A	35	p.Tyr2608X	De novo	41
c.7879C>T	36	p.Arg2627X	ND	42
c.7884_7885delTA	36	p.His2628fs	De novo	43
c.8016G>A	37	p.Trp2672X	ND	
c.8744_8745dupG	38	p.Leu2916fs	ND	44

*Nomenclature according to http://www.genomic.unimelb.edu.au/mdi/mutnomen/; †recurrent mutation.
excl. excluded; Mat, Material; ND, not done.

additional information from clinicians, resulted in a description of the clinical features of the 47 selected patients as outlined in tables 3 and 4. Details of these features are provided below. All 47 cases were included in the evaluation unless stated otherwise. The diagnostic criteria of Blake and Verloes (table 1) could be applied to 38 cases.[9 11] There was

Table 3 Anomalies in 47 *CHD7* positive patients

Patient	Sex	Age at clinical evaluation	Mental retardation	Neurological abnormalities	Skeletal abnormalities	Urogenital findings	Gonadotrophin deficiency	Diagnostic criteria (table 1)	
								Blake[9]	Verloes[11]
1	F	17	+++				+	+	
2	F	19	?				+		
3	F	17	+				+	+	+
4	F	6	+					+	+
5	F	6	+++						
6	M	10	−					+	+
7	F	11	++		S			+	+
8	M	7	+++			HK		+	+
9	M	4	?						
10	M	<1	?					+	+
11	F	<1	?			C		+	
12	M	Day 6*	?	AC+CH	S+HV		+		
13	M	32	+++		HV		+	+	
14	F	35	++	C	S		+	+	+
15	F	15	+					+	+
16	M	19	−		S		+	+	+
17	M	19	++	ACC	S	R	+	+	+
18	M	Day 12*	?					+	+
19	F	5	+	H				+	
20	M	Month 5*	?	C		A			
21	M	40	+++				+	+	+
22	F	22	++	H + C		R	+	+	+
23	M	19	−					+	+
24	F	Month 6*	?						
25	M	1	?					+	+
26	F	11	++					+	+
27	F	Day 2*	?						
28	F	5	+					−	−
29	M	7	++					+	
30	M	3	+++					+	
31	M	Year 14*	++					+	+
32	F	12	++		K			+	+
33	M	Week 5*	?						+
34	M	6	?						
35	M	6	?			HK	+	+	+
36	M	20	+++	C			+	+	+
37	M	<1	?		T	R			
38	F	Day 21*	?			HK			+
39	F	3	−			A		+	+
40	M	20	+++		S		+	+	+
41	F	10	+++					+	+
42	F	<1	?						
43	F	26	−				+	+	+
44	F	20	−				+	+	+
Missense mutations									
45	F	15	−					+	+
46	F	15	+					+	+
47	F	16	−	C	HV			+	

*Deceased.
Mental retardation: −: normal intelligence; +: mild MR; ++: moderate MR; +++: severe MR.
Neurological abnormalities: AC, agenesis of corpus callosum; ACC, atrophy of cerebral cortex; C, convulsions; CH, cerebellar hypoplasia; H, hydrocephaly.
Skeletal abnormalities: HV, hypoplastic vertebrae; K, kyphosis; S, scoliosis; T, triphalangeal thumb.
Urogenital anomalies: A, agenesis of one kidney; C, renal cysts; HK, horseshoe kidney; R, reflux.

only one patient who did not fulfil either set of diagnostic criteria.

Sufficient clinical information could also be obtained for 23 of the 38 *CHD7* negative patients; only two of these patients fulfilled the clinical diagnostic criteria of Blake and Verloes.[9 11]

A summary of all clinical data of the 47 *CHD7* positive patients is given below. A detailed case report is then provided of a girl who did not fulfil the diagnostic criteria of Blake and Verloes (table 1)[9 11]; the intra-familial variability in sib pairs is also delineated.

Neonatal period

The median gestational age of the patients was 38.2 weeks (n = 45, range 30–42 weeks). Only one patient was reported to be small for gestational age, while feeding difficulties were reported in 33 (70%) patients. Four patients required a gastrostomy due to severe feeding problems.

Four patients died during the neonatal period, three during the first half year of life, and one at the age of 14 years. At the time of investigation four patients were below the age of 1 year.

Coloboma of the eye

A coloboma of one (n = 4) or both (n = 29) eyes was present in 33 patients (70%). As the iris was involved in only nine patients, the coloboma was only visible by fundoscopy in most patients. In none of the patients was the coloboma restricted to the iris only. Microphthalmia was present in ten patients (21%).

Congenital heart defects

Thirty one (66%) patients had a congenital heart defect. Fourteen (30%) patients had major heart defects: six tetralogy of Fallot, two double-outlet right ventricle (one combined with

Table 4 Further anomalies in *47 CHD7* positive patients

Pat.	Sex	Age at clinical evaluation	Gestation (weeks)	Birth weight (g)	Coloboma		Microphthalmia	Heart defect	Atresia of choanae	Height	Micropenis/ cryptorchidism	External ear anomaly	Hearing loss	Vestibular dysfunction	Facial nerve palsy	Oesophageal fistula	Cleft lip	Cleft palate
					Left	Right												
1	F	17	38	2450	IRCO	IRCO	L/R	−	−	<P3		+	+	?	−	−	−	−
2	F	19	41	3520	RC	RC	R	+	−	?		+	+	?	−	−	−	+
3	F	17	38	2435	−	−	−	+	+	<P3		+	−	+++	−	−	−	−
4	F	6	32	1805	−	−	−	+	−	<P3		+	+	+++	−	+	+	+
5	F	6	36	?	IRCO	IRCO	−	+	−	?		+	?	?	−	−	+	+
6	M	10	38	3025	−	−	−	−	−	P3	+	+	+	+++	+	−	−	+
7	F	11	40	3350	CRO	CRO	L/R	−	−	?		+	+	+++	−	−	−	+
8	M	7	39	2530	CRO	O	L	−	−	<P3	+	+	+	+++	−	−	−	−
9	M	4	39	3200	−	−	−	−	−	>P10	+	+	+	?	−	−	+	+
10	M	<1	31	1650	−	−	−	−	+	?	+	+	+	+++	−	−	−	−
11	F	<1	40	4082	R	R	−	+	−	?		+	+	?	−	−	+	+
12	M	Day 6*	38	3060	IRO	RO	−	+	−	?	−	+	?	?	−	−	+	+
13	M	32	37	2900	−	−	−	−	+	<P3	+	+	+	?	−	−	−	−
14	F	35	?	3250	C	−	L	+	+	<P3		+	+	+++	−	−	−	−
15	F	15	39	2910	−	−	−	+	+	<P3		+	+	++	−	−	−	−
16	M	19	42	3700	IRC	IRC	−	−	−	P15	+	+	+	++	−	−	−	−
17	M	19	40	2870	−	−	−	+	+	<P3	+	+	+	+	−	−	−	−
18	M	Day 12*	35	2250	IO	O	−	+	+	?	+	+	+	+++	−	−	−	−
19	F	5	40	3840	RO	RO	−	+	−	<P3		+	−	?	−	−	+	+
20	M	Month 5*	38	3408	−	−	−	+	+	?	+		?	?	−	−	−	−
21	M	40	40	3250	RO	RO	R	+	−	P10	+	+	+	?	+	+	−	−
22	F	22	36	2000	−	−	−	+	+	<P3		+	+	+++	−	+	−	−
23	M	19	42	2800	IR	R	−	+	−	<P3	+	+	+	+++	−	−	+	+
24	F	Month 6*	42	3450	IR	IR	−	+	−	?		+	+	?	+	−	−	−
25	M	1	37	2940	R	R	−	+	−	P50	+	+	+	+++	+	+	−	−
26	F	11	35	1910	RC	IRC	−	+	+	<P3		+	+	+	−	−	−	−
27	F	Day 2*	35	1500	−	−	−	+	+	?		+	?	?	−	+	−	−
28	F	5	40	3700	−	−	−	+	−	P3		+	+	+++	−	−	−	−
29	M	7	40	2867	−	−	−	+	−	<P3	+	+	−	?	+	−	+	+
30	M	3	38	3440	RC	−	−	−	−	P3	+	+	+	?	−	+	−	−
31	M	Year 14*	?	3500	RC	RC	−	−	−	<P3	−	+	+	+++	−	+	+	+
32	F	12	41	2800	IR	R	L	+	−	<P10		+	+	+++	+	−	−	−
33	M	Week 5*	30	1470	RO	RO	U	−	+	?	−	+	+	?	−	+	−	−
34	M	6	37	2700	R	R	−	+	−	?	−	+	+	?	−	−	−	+
35	M	6	35	2835	O	R	R	+	−	<P3	+	+	+	+++	+	−	−	+
36	M	20	40	2820	R	R	−	−	+	<P3	+	+	+	?	−	−	+	−
37	M	<1	39	3114	R	R	−	+	−	?	+	+	?	?	−	−	−	−
38	F	Day 21*	35	2393	CRO	CRO	−	+	+	?		+	?	?	−	−	−	−
39	F	3	40	3340	CR	CR	−	+	−	<P3		+	+	+++	−	−	−	−
40	M	20	42	3490	CR	CR	−	+	−	<P3	+	+	+	+	−	−	+	+
41	F	10	37	2650	−	−	−	+	+	<P3		+	+	+++	−	−	−	−
42	F	<1	41	2830	CO	O	−	+	−	?		+	+	?	+	−	−	−
43	F	26	40	2450	R	−	−	+	−	<P3		+	+	+++	+	−	−	−
44	F	20	36	2450	CRO	CRO	−	−	+	?		+	+	+++	+	−	+	+
Missense mutations																		
45	F	15	38	2980	O	O	L/R	−	−	<P3		+	+	+++	−	−	−	−
46	F	15	41	2500	C	C	−	−	−	P10		+	+	+++	−	−	−	−
47	F	16	40	2100	C	−	−	−	+	P3		+	−	+	−	−	−	−

*Deceased.
Coloboma: C; choroidea; I, iris; O, optic disc; R; retina.
Microphthalmia: L, left; R, right; U, unilateral, side not known.
Vestibular dysfunction: +: history of unsteadiness; ++: vestibular areflexia; +++: semicircular canal agenesis on CT scan of inner ear.
Pat., patient

hypoplastic left heart and AVSD), three isolated hypoplastic left heart syndrome, one hypoplastic right heart syndrome, one agenesis of the pulmonary valve combined with hypoplastic left heart, and one Shone's complex. A right descending aorta was present in three patients and one patient had a vascular ring. The other patients had solitary patent ductus arteriosus beyond infancy (n = 3), patent ductus arteriosus combined with atrium septum defect, and/or ventricular septum defect (n = 6) or a solitary septal defect (n = 4).

Retardation of growth and development

A height below the third percentile was reported in 21 out of 32 patients (63%).

Speech development varied from mild speech delay to severe retardation without speech. Learning disabilities were reported in 24 (75%) out of 32 patients who were above the age of 12 months at last examination. Eight patients (25%) had no cognitive impairment.

Endocrine and urogenital abnormalities

At the time of *CHD7* testing, 15 patients (eight girls, seven boys) were over 15 years of age. Gonadotrophin deficiency was present in seven (88%) of these girls, and six (86%) of these boys. Two girls had their menarche at age 14. A hypoplastic uterus was found by ultrasound investigation in three girls. Of all 22 mutation positive boys, four (18%) had cryptorchidism, six (27%) had micropenis, and seven (32%) had both cryptorchidism and micropenis.

Three patients had a horseshoe kidney and in two patients agenesis of the left kidney was demonstrated. A vesicoureteral reflux was reported in three patients and one patient had renal cysts.

Ear and vestibular abnormalities

Dysmorphisms of the ears were noted in all patients, ranging from typical CHARGE ears (small, square, low set, and protruding) to minor structural abnormalities such as absence of an earlobe. One patient had a pre-auricular pit and one patient had narrow external auditory canals. Hearing impairment was demonstrated in 37 out of 41 patients (90%). In 27 patients severe bilateral hearing impairment was observed, while five patients showed asymmetric hearing impairment with unilateral normal or mild hearing loss.

In all 21 patients who underwent CT scanning of the temporal bones, agenesis of the semicircular canals was demonstrated. Vestibular areflexia was demonstrated in two more patients and four patients had a history of balance disturbances. In total, therefore, 27 patients (57%) had some evidence of vestibular anomaly. However, information on this subject was not available for the remaining patients, although motor delay (possibly due to vestibular areflexia) was present in all cases on direct questioning.

Nasopharyngeal abnormalities and clefting

Choanal atresia was present in 17 patients (36%) and was unilateral in only three of them.

Respiratory insufficiency during the neonatal period was reported in 24 patients (51%), 22 of whom had either choanal atresia or a congenital heart defect or both. Tracheomalacia was present in one patient.

Clefting was present in 17 patients (36%): 11 had a cleft lip and palate, five had an isolated cleft palate, and one had an isolated cleft lip.

Gastrointestinal abnormalities

Eight patients (17%) had oesophageal atresia, which in three was accompanied by a tracheo-oesophageal fistula. Two patients had a diaphragmatic hernia and one anal stenosis.

Figure 2 Patient 28, who is *CHD7* mutation positive but does not fulfil the diagnostic criteria (see text). (Written consent was obtained for the publication of this picture.)

Neurological abnormalities

A minority of patients (n = 4, 9%) had central nervous system abnormalities, including corpus callosum agenesis combined with cerebellar hypoplasia (n = 1), hydrocephaly (n = 2), and atrophy of the cerebral cortex (n = 1). Five patients had convulsions.

Facial nerve palsy was present in 10 patients (21%) and mostly (nine out of 10) involved the right-sided facial nerve.

Skeletal abnormalities

Scoliosis was demonstrated in six patients (13%), kyphosis in one, and abnormalities of the vertebral bodies in three (6%). In one patient a triphalangeal thumb was demonstrated.

Aspecific CHARGE syndrome

Patient 28 (born at 40 weeks' gestation; birth weight 3700 g, 70th centile, fig 2) was a 5 year old girl with developmental delay, slightly dysmorphic ears, and severe hearing impairment. CT scan showed bilateral agenesis of the semicircular canals. She required a gastrostomy due to severe feeding problems and had surgery on a congenital vascular ring. Her height was at the third centile. No choanal atresia, cleft palate, or coloboma could be detected. In this girl, the only individual in our *CHD7* positive series who did not fulfil the current diagnostic criteria for CHARGE syndrome (table 1),[9 11] a de novo nonsense mutation was identified, 5833C>T (R1945X) in exon 29 of *CHD7*.

Familial cases

Two sib pairs were included from two families. In both cases, identical *CHD7* mutations were identified in the two sibs. Interestingly, in both cases the affected sib pairs showed distinct clinical features.

Sib pair 1 were monozygotic twin sisters born at 35 weeks' gestation (patients 26 and 27; tables 3 and 4, fig 3A,B). Patient 27, who had a birth weight of 1500 g (5th–10th centile), died 29 h after birth due to a combination of hypoplastic left heart syndrome and bilateral choanal atresia. She also had a tracheo-oesophageal fistula and typical CHARGE ears. A hearing test was not performed. There were no colobomata of the irides.

Patient 26 had a birth weight of 1910 g (25th centile). When examined at the age of 12 years, she had short stature (<3rd centile) and was functioning 4 years behind her chronological age. She was born with a large patent ductus arteriosis that required surgery and she needed numerous procedures to correct bilateral choanal atresia. Her first years of life were complicated by feeding problems, for which she had a gastrostomy until the age of 6 years. She had severe bilateral deafness, abnormal external ears like her twin sister, and bilateral chorioretinal colobomata with a right-sided iris coloboma and an unusual inferior pigment pattern in her left iris. Agenesis of the semicircular canals was not tested for by CT scan, but her gait was unsteady.

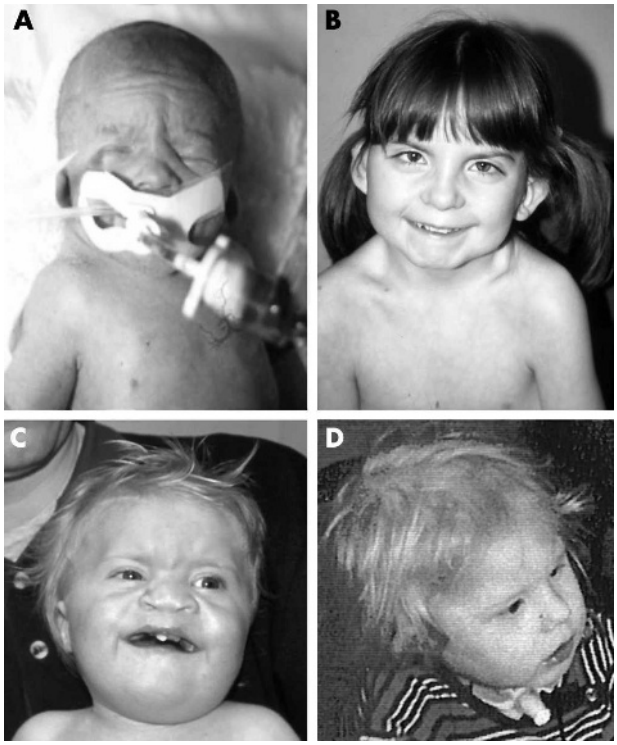

Figure 3 (A) Twin 1 of sib pair 1 (patient 27 in tables 3 and 4), who died shortly after birth; (B) twin 2 of sib pair 1 (patient 26 in tables 3 and 4) at the age of 7 years; (C) sib 1 and (D) sib 2 of sib pair 2, both at the age of 2 years (patients 29 and 30 in tables 3 and 4). (Written consent was obtained for publication of these photographs.)

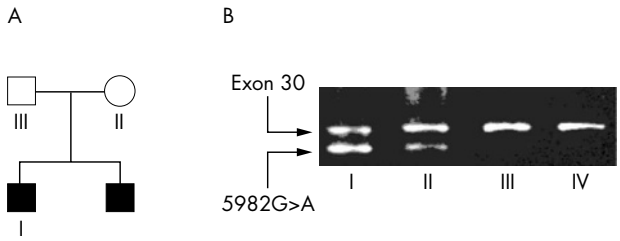

Figure 4 (A) Results of allele specific PCR for the 5982G>A mutation in the family with two affected boys. (B) DNAs of the indicated family members and an unrelated unaffected control (IV) were subjected to a multiplex PCR using a mutation specific primer (5982G>A, lower band) and the regular primer set for exon 30 (upper band). The mutation found in the boys (I) was also present in the mother (II). The different relative amounts of the fragments of individual I and II might reflect the presumed mosaicism in the mother.

Zygosity testing with five unlinked markers was performed and the results were consistent with the twins being monozygotic. In both sisters the spectrum of congenital anomalies was caused by an insertion 5752_5753insA in exon 29 of *CHD7*.

Sib pair 2 consisted of two brothers (patients 29 and 30; tables 3 and 4, fig 3C,D). Patient 29 was 7 years of age (born at 40 weeks' gestation; birth weight 2867 g, 10th centile). He had surgery for cleft lip/palate and a complex heart defect (DORV, AVSD, hypoplastic left heart). He had short stature (3rd centile) and severe developmental delay. His ears showed the typical CHARGE dysmorphisms and he had bilateral hearing loss and unilateral facial nerve palsy. He had no colobomata or choanal atresia.

Patient 30, who was 4 years younger, had bilateral hearing loss and typical CHARGE ears, a coloboma of the left retina and choroid, and underwent surgery for a tracheo-oesophageal fistula. He had short stature (<3rd centile) and was severely mentally retarded. He had vocal cord palsy. This boy had no heart defect and no choanal atresia.

Both brothers have a 5982G>A (W1994X) mutation in exon 30 of CHD7. Sequence analysis of both parents revealed no mutation in the father and a minor aberrant peak in DNA extracted from lymphocytes of the mother. This indicated that a possible mosaicism was present in the mother. This was further investigated and confirmed by an allele specific PCR, using a primer carrying the 5982G>A mutation at the 3′ end, in combination with the regular exon 30 primer set (fig 4). Clinical examination of the mother did not reveal any signs of CHARGE syndrome.

DISCUSSION

At the time of evaluation of our clinical data, *CHD7* sequencing had been performed in 107 index patients referred to our laboratory because of clinical features suggestive of CHARGE syndrome. Pathogenic mutations were identified in 69 patients (65%), including six patients who had previously tested negative.[12] All mutations except two were unique and most mutations had a severe effect on the *CHD7* protein, being either nonsense or frameshift mutations (70%).

From both our previous data and a recent report by Arrington *et al*, it is known that microdeletions of the chromosome 8q12.1 region, including the *CHD7* gene, may also result in CHARGE syndrome.[12 15] We excluded the presence of such microdeletions in the patients without *CHD7* mutations by MLPA. From these results we conclude that whole gene deletions of the *CHD7* gene are not a frequent cause of CHARGE syndrome. Currently, we are extending our MLPA analyses in order to assess for the presence of small intragenic deletions.

In 20 out of 21 families a de novo occurrence of the *CHD7* mutation could be proven. In the mother of the sibs with the 5982G>A(W1994X) change, this mutation was present as a somatic mosaicism. It is likely that germline mosaicism exists as well. As a consequence, prenatal diagnosis should be offered to all parents of children with an apparently de novo *CHD7* mutation.

Of the 69 *CHD7* mutation positive patients, 45 index cases were selected for further clinical study together with two sibs, resulting in a cohort of 47 patients. Due to a short follow-up period, clinical information was limited in 11 patients, especially regarding hearing, growth, and development. From the data presented in tables 3 and 4 and the detailed clinical description of our patients, it is clear that within the *CHD7* mutation positive subset of CHARGE patients an extensive variability in clinical presentation exists, without any obvious genotype-phenotype correlation. This is best demonstrated in the two sib pairs. In the first sib pair, both twin girls had choanal atresia and a heart defect, but they were discordant for the coloboma and tracheo-oesophageal fistula. The boys of the other sib pair were discordant for cleft lip/palate, heart defect, tracheo-oesophageal fistula, coloboma, and hearing loss.

Missense mutations were found in three patients of the clinical study group, one of whom was mildly mentally retarded. The other two had normal levels of intelligence. However, normal intelligence was also present in five patients with a nonsense mutation. Overall clinical comparison of these three patients with a missense mutation with the rest of the study group did not reveal any clear differences. However, it is still possible that less severe mutations (that is, missense mutations) result in a less specific phenotype, not recognised as CHARGE syndrome.

Table 5 The frequencies of characteristic CHARGE findings in a population of *CHD7* positive patients compared to the literature

	This study, n=47		Stromland *et al*,[16] n=30	Issekutz *et al*,[10] n=77	Tellier *et al*,[3] n=47
	n	%	%	%	%
Coloboma	33/47	70	90	77	79
Choanal atresia	17/47	36	35	64	57
Ear anomaly/deafness	47/47	100	90	96	100
Cranial nerves	10/47*	21	32*	91	78
Genital hypoplasia (males)	17/22	77	67	65	53
Heart defects	31/47	66	52	84	85
Cleft lip/palate	17/47	36	19	18	17
TE fistula	8/47	17	16	19	15
Growth deficiency	21/32	66	48	58	75
Renal	9/47	19	12	36	19
Spine	9/47	19	27	9	13

*Data on facial nerve only.

Hence, such patients may not be included in this study. On the other hand, patients with a *CHD7* deletion may be more severely affected than patients with a *CHD7* mutation, especially if multiple adjacent genes are deleted. Further studies are needed to explore this.

In table 5 the frequency of the main features of CHARGE syndrome in our group of *CHD7* mutation positive patients is compared with data from the literature.

The distribution of features in the clinically diagnosed CHARGE syndrome patients as reviewed by Stromland *et al*, Issekutz *et al*, and Tellier *et al*, is comparable to that in our *CHD7* mutation positive patients.[3 10 16] This indicates that, within the patient group that fulfils the clinical diagnosis of CHARGE syndrome, there is not a specific subgroup that is more likely to have a *CHD7* mutation. None of the clinical features seems to be obligatory for a *CHD7* mutation, with the possible exception of vestibular anomalies. Several reports have stressed the high frequency and the high specificity of anomalies of the semicircular canals.[1 2 17 18] This was also observed in our cohort of patients. All patients investigated by CT scan or vestibular function tests had either abnormal function or an aplasia of the semicircular canals.

The effect of a *CHD7* mutation on a specific organ is variable and does not predict the consequences for other organ systems in which *CHD7* is expressed. For instance, a severe heart defect does not exclude normal intelligence (for example, individual 43, tables 3 and 4) and severe mental retardation does not have to be accompanied by severe defects in other organs (for example, individual 8, tables 3 and 4). This results in enormous clinical variability, even within sib pairs.

We carefully tested whether both sets of diagnostic criteria could be applied to our patients (tables 3 and 4).[9 11] This was not possible in all cases, since, for example, CT scanning of the temporal bones is required in order to apply the diagnostic criteria proposed by Verloes. For simplification we decided to use the 1998 Blake criteria as listed in table 1 instead of the refined criteria adopted for different age groups.[10] Both Blake and Verloes require that at least a coloboma or choanal atresia is present for the diagnosis CHARGE syndrome. Five patients in our study group (individuals 4, 6, 9, 28, and 29 in tables 3 and 4) had neither coloboma nor choanal atresia. Blake *et al* argued that the choanae are usually patent when orofacial clefting is present and palatal clefting can be substituted for choanal atresia in the scoring criteria.[9] As a consequence, only one patient (individual 28 in tables 3 and 4) failed to fulfil the diagnostic criteria for CHARGE syndrome according to both Blake and Verloes. In 38 patients with features suggestive of CHARGE syndrome, no *CHD7* mutation and/or deletion was identified. For 27 of these patients, sufficient clinical data were available to apply the clinical diagnostic criteria. Only two of these 27 *CHD7* mutation negative patients fulfilled the diagnostic criteria. In both patients aplasia of the semicircular canals was demonstrated. As a consequence, the positive predictive value of the clinical diagnostic criteria is 36/38 (95%). This is substantiated by the fact that after improvement of the sequencing procedure (see supplemental table I available at http://www.jmedgenet.com/supplemental), the mutation positive percentage in our first reported cohort reaches 95% (18/19).[12] In the context of the previously suggested genetic heterogeneity,[19–21] this is an interesting observation that needs confirmation.

We would like to stress that CHARGE syndrome remains a clinical diagnosis. Although the high percentage of *CHD7* mutations in clinically diagnosed CHARGE syndrome patients indicates that *CHD7* is the major gene involved, this diagnosis cannot be rejected based on absence of a *CHD7* mutation. On the other hand, based on the clinical criteria alone, one *CHD7* positive patient would have been missed in our series.

In conclusion, we confirm that mostly unique *CHD7* mutations account for the majority of cases with CHARGE syndrome, with a broad clinical variability and without an obvious genotype-phenotype correlation. In addition, we provided evidence for germline mosaicism.

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ELECTRONIC-DATABASE INFORMATION

The MRC-Holland website is at www.mlpa.com; and the HGVS Nomenclature for the description of sequence variations website is at http://www.genomic.unimelb.edu.au/mdi/mutnomen

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